REVIEW ARTICLE

Chlamydial Zoonoses

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SUMMARY

Background: Zoonoses were already a subject of intense interest even before the SARS and avian influenza epidemics arose. For many years, chlamydiae have been hypothesized to be important zoonotic pathogens, because of their wide distribution and their infectious cycle. This article provides an overview of the current state of knowledge on this subject.

Methods: The authors present a selective review of the literature as well as their own findings.

Results: The scientific knowledge of the distribution and infectious cycle of chlamydiae is still inadequate. The laboratory diagnosis of chlamydial zoonoses remains unsatisfactory in both human and veterinary medicine, as there are no commercially available sensitive and species-specific tests. Acute chlamydial infections are usually treated with macrolides, tetracyclines, or quinolones. Persistent varieties are not covered by standard therapy.

Conclusions: There is a considerable need for research on chlamydial infections, especially with regard to the diagnosis and treatment of persistent varieties.

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Zoonoses are infectious diseases which can be transmitted from animals to man. The best known zoonotic pathogen is currently *Chlamydia psittaci*, the pathogen of ornithosis. Although there have been many published reports about outbreaks, the exact prevalence or incidence is unclear (1). *Chlamydia trachomatis* is the most frequent bacterial pathogen of sexually transmissible diseases; its incidence in Europe lies between 2% and 17% in women with asymptomatic disease (2). *Chlamydia trachomatis* is also a pathogen of trachoma (keratoconjunctivitis). 84 million people in the world suffer from this infection and 1.3 million of these have become blind as a result (3). *Chlamydophila pneumoniae* is recognized as a pathogen of community-acquired pneumonia and can be identified in ca. 10% of cases (4).

Chlamydiae are among the most prevalent microorganisms in the animal kingdom. The most prominent examples are enzoonotic ovine abortion from *Chlamydia abortus* and ornithosis from *Chlamydia psittaci*, as already mentioned.

The aim of the present overview is to present human diseases from chlamydiae, in the context of their zoonotic potential, current knowledge and perspectives in diagnostic testing and therapy, as well as current approaches in research. The literature search was performed in the database PubMed, with the key words “chlamydia” and “zoonotic.”

Chlamydiae as infectious pathogens

Chlamydiae are Gram-negative, obligatory intracellular bacteria *(Figure 1)* and have a broad host spectrum. They are clinically and epidemiologically important throughout the world, both in human and in veterinary medicine.

Human pathogenic chlamydiae typically cause infections of the eye, or of the urogenital or respiratory tracts. These infections are often initially not recognized or misinterpreted, as they are difficult to diagnose and the symptoms are mild. Chronic and repeated infections may lead to irreversible damage, including blindness (trachoma) after infection of the eye and tubal infertility after infection of the female genital tract.

Complications after infection—most importantly reactive arthritis—are presumably linked to the immunological response. Moreover, chlamydiae have been linked etiologically to a series of chronic inflammatory

processes, particularly arteriosclerosis. However, this is still controversial. The *Table* gives an overview of the significance of the different Chlamydia species and the spectrum of disease.

Chlamydia infections can lead to a notably wide variety of clinical manifestations. This may be linked to both the specific properties of the pathogen itself and the individual susceptibility of the affected patient.

Under ideal growth conditions, chlamydiae pass through a two-step developmental cycle within 2 to 3 days. The first step is that the infectious, but metabolically inactive, coccoid elementary bodies penetrate into the host cell and form vacuole-like inclusions *(Figure 2)*. Within these inclusions, the conversion is completed to metabolically active, but non-infectious, pleomorphic reticular bodies, which replicate by binary fission. Condensation and conversion then occur within the elementary bodies, which are released after rupture of the inclusion and can then infect new cells. This completes the cycle.

This developmental cycle can be influenced by the host's immune response (for example, increased INF-γ production), by adverse external conditions, such as the lack of essential substrates (for example, iron, or amino acids such as tryptophan), or by antibiotic therapy. The chlamydiae react to these influences by passing into the persistent state, which is characterized by aberrant (enlarged) morphology of the reticular body. It is thought that this ensures the long-term survival of the Chlamydia cell within the host cell. On the other hand, Chlamydiae, as intracellular microorganisms, can influence the cell cycle, metabolism and antigen presentation of the host cell.

It has been known for many years that *Chlamydia (C.) trachomatis* causes the most common sexually transmissible diseases in the world and that *Chlamydophila/Chlamydia pneumoniae* is a pathogen of human respiratory infections; these microorganisms have therefore attracted much clinical and scientific attention. On the other hand, the significance for human medicine of the veterinary species *Chlamydophila (Cp.) abortus*, *Cp. pecorum*, *Cp. felis*, *Cp. caviae*, and *C. suis* remains unclear and relevant studies have just been started.

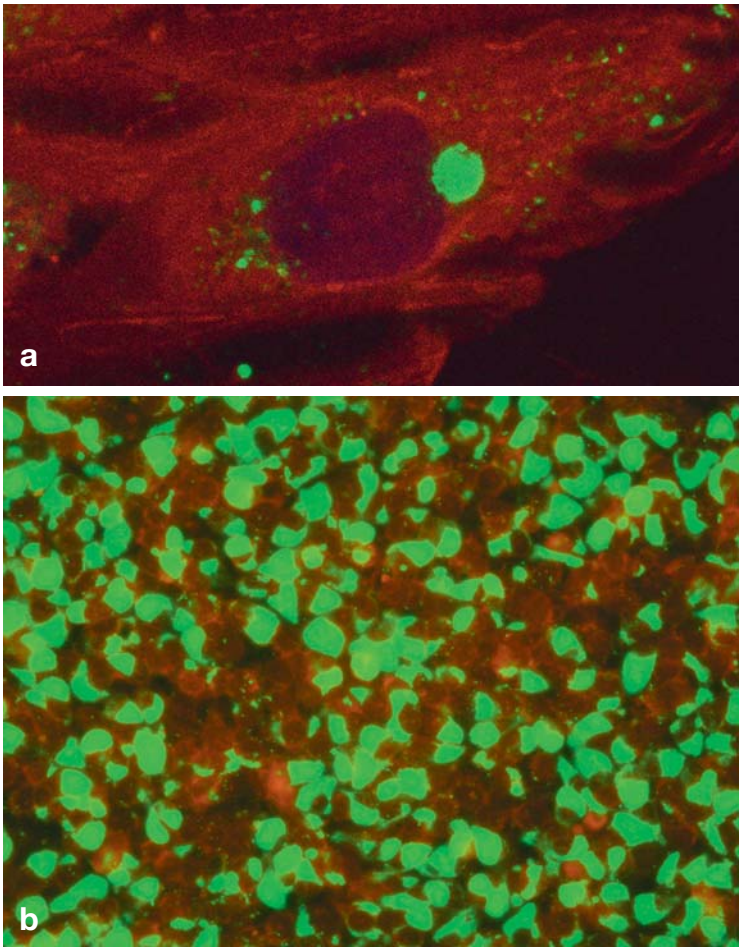


Figure 1: a) Smooth muscle cell with inclusion bodies of *Chlamydophila (Cp.) pneumoniae* (image: Dr. J. Rödel); b) cell culture (BGM), infected with *Cp. psittaci*, strain DC15, stained with a fluorescently labeled LPS antibody; the chlamydial inclusion appear green (image: Dr. E. Schubert)

Human diseases of zoonotic origin

Human psittacosis is also known as ornithosis or parrot disease and is a relatively rare zoonosis, caused by *Chlamydophila (Cp.) psittaci*, formerly known as the avian serotype of *Chlamydia psittaci*. The clinical course of this condition is usually severe.

The disease was initially linked to keeping pet birds (Psittacidae; true parrots), although there is a major risk of human infection from working with domestic poultry or wildfowl (for example, in the poultry processing industry) (5, e1). In the pre-antibiotic era, psitaccosis was fatal in 15% to 20% of cases. Contacts with infected birds and their excretions can be documented in up to 62% of human patients (e2). It is believed that the pathogen is transmitted through aerosols. The

TABLE

Diseases caused by Chlamydiae in man and in animals

Genus	Species	Diseases in man	Frequency	Diseases in animals
Chlamydia	muridarum	Non-pathogenic in man		
	suis	Non-pathogenic in man		Pneumonia, enteritis, conjunctivitis (pig)
	trachomatis	Urogenital infections Adnexitis Reactive arthritis Swimming pool conjunctivitis Neonatal conjunctivitis Neonatal pneumonia	Frequent in adolescents Occasional Occasional Rare Rare Rare	Unknown
	trachomatis, trachoma biovar	Trachoma	Frequent in tropical countries	Unknown
	trachomatis, LGV biovar	Venereal lymphogranuloma	Occasional in tropical countries, also in HIV-positive men in Europe	Unknown
Chlamydophila	abortus	Septic infection Abortion	Rare	Enzootic ovine abortion, also occasionally abortion in cattle
	caviae	Non-pathogenic in man		Conjunctivitis (guinea pig)
	felis	Conjunctivitis	Rare	Keratoconjunctivitis (cat)
	pecorum	Non-pathogenic in man		Enteritis, abortion, conjunctivitis, pneumonia, encephalomyelitis, polyarthritis (ruminants)
	pneumoniae, TWAR biovar	Respiratory infections Atypical pneumonia Reactive arthritis Infestation of the arteriosclerotic plaque	Very frequent Occasional Occasional Probably frequent	Uncertain
	pneumoniae, koala biovar	Non-pathogenic in man		Uncertain
	pneumoniae, equine biovar	Non-pathogenic in man		Uncertain
	psittaci	Respiratory infections Pneumonia Myocarditis Hepatitis Encephalitis	Occasional Occasional Rare Very rare Very rare	Psittacosis/ornithosis (true parrots, domestic poultry)
Simkania	negevensis	Respiratory infections	Probably frequent	Unknown
Parachlamydia	acantamoebae	Non-pathogenic in man		Under study
Waddlia	chondrophila	Non-pathogenic in man		Under study

elementary bodies of the pathogen are thought to be environmentally resistant. Thus, in view of the danger of human infection (6), the avian strains of *Cp. psittaci* have been classified into risk group 3 in the German Biosubstance Regulations.

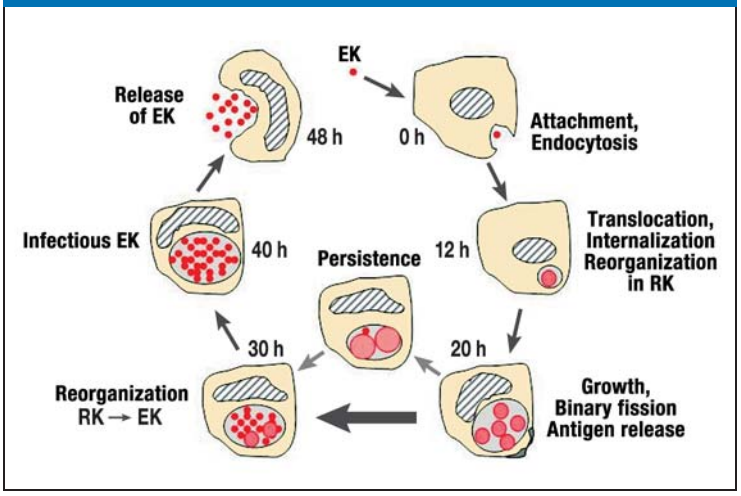
The initial symptoms of ornithosis resemble those of influenza, including fever of up to 40°C, headache, exhaustion, arthralgia, and loss of appetite. Symptoms of pneumonia develop towards the end of the first week of the disease. These are mostly only detectable in x-rays (atypical pneumonia), but may be extensive and exhibit carnification (e3). *Figure 3* shows the chest x-ray of an ornithosis patient (7). There have, however, also been reports of systemic general infections, with the complications of endocarditis, myocarditis, glomerulonephritis, hepatitis, pancreatitis, or meningoencephalitis (e4, e5).

This is a notifiable disease in Germany. The annual number of cases is decreasing and has lain between 15 and 156 during the last eight years, including several deaths. On the other hand, it is unclear whether this statistical decrease reflects a real decrease in the number of infections, or whether it is partially linked to inadequate diagnostic testing, as there are regularly risks from local (8, e6) and international (e7, e8) outbreaks of psittacosis. The specific history of contacts with animals and, particularly, birds is thus of decisive importance for rapid diagnosis.

Another important and potentially fatal zoonosis is enzootic ovine abortion from the pathogen *Cp. abortus* (formerly, *Chlamydia psittacosis*, serotype 1). Depending on the degree of infestation and purchasing practices, this infection can cause abortion in as many as 80% of ewes in affected stocks and can lead to serious economic problems in animal husbandry in affected areas (9). Moreover, infected animals are a potential risk for pregnant women. Thus, there have been several reports on spontaneous abortions and potentially fatal systemic infections in pregnant women after exposure to infected sheep or goats (10, e9–e12). If a woman with pregnancy complications works in agriculture and/or has contact to pregnant animals in her medical history, it is absolutely essential that *Cp. abortus* should be included in the differential diagnosis. It may also be necessary to consult a specialized laboratory.

Aside from acute infections with chlamydiae, chronic persistent infections have been described. Recurrent or long-term exposure to *Cp. pneumoniae* has been associated with asthma symptoms (11) and chronic infection is thought to contribute to the development of obstruction of the respiratory tract in non-atopic bronchial asthma (e13). Virtually nothing is known about whether other Chlamydia species, particularly zoonotic chlamydiae, can evoke similar diseases. Macrolides are antibiotics used for the treatment of atypical bacterial infections, including Chlamydiae; current data demonstrate that they significantly reduce asthma symptoms in patients with acute exacerbations. The mean difference in the decrease in symptom scores was –0.3, with a 95% confidence interval of between

FIGURE 2



Development cycle of Chlamydia; the values given are approximate and may vary between strains; EK, elementary body; RK, reticular body

–0.5 and –0.1 (p = 0.004) (12). In another study, 42 patients with chronic obstructive lung disease (COPD) were investigated. Using the polymerase chain reaction (PCR), Chlamydiae were detected in resected lung tissue in 15% of the cases (e14) and in induced sputum in 24% of cases (13). Chlamydiae were also detected in lung emphysema, using direct immunofluorescence, electron microscopy and PCR (14, e15). Moreover, it was shown that antibody formation was related to the severity of the pulmonary emphysema (e16). No significant difference between Chlamydia-positive and -negative patients was found with respect to the local inflammatory reaction in lung sections of COPD patients (e14). These preliminary data indicate that the pathogen is associated with chronic diseases of the respiratory tract, even though the mechanisms are not yet understood. Data from studies on large animals with spontaneously acquired Chlamydia infections support the thesis that persistent or recurrent Chlamydia infections are associated with chronic airway obstruction (15).

There is an occupational risk for persons with direct contact to infected animals, leading to high levels of exposure (e17). On the other hand, there is also ecological exposure to wild birds, particularly city pigeons, and this can lead to infections of the respiratory tract (18e).

Nevertheless, most published articles have been case reports and there are no available systematic data on the epidemiological situation in Europe or on the zoonotic risk of specific groups of individuals.

Current scientific knowledge on chlamydial zoonoses is unsatisfactory, essentially for the following reasons:

- Available laboratory diagnostic testing is mostly incapable of supporting a clear diagnosis and the timely initiation of therapy with suitable drugs.
- Chronic or persistent Chlamydia infections cannot be detected with the available methods.

Figure 3: Chest x-ray of an ornithosis patient (aged 73) with dense infiltration in the left lower lobe and hilus enlargement. Symptoms: flu-like symptoms, dyspnea, dry cough with hemoptysis, fever up to 39°C. In auscultation, fine rales left basal. The pathogen *Cp. psittaci* was detected in culture and by PCR from BAL (7). PCR, polymerase chain reaction; BAL, bronchoalveolar lavage

- Genetic modification of Chlamydia is currently impossible.

Zoonotic potential of chlamydiae from cow and pig stocks

Molecular diagnostic methods, such as PCR and DNA microarray techniques, have now been applied to cow and pig stocks. These have not only detected species-adapted chlamydiae (such as *Cp. pecorum* in the cow and *Chlamydia suis* in the pig), but also surprisingly high levels of *Cp. abortus* and *Cp. psittaci*. The presence of these species in other host animals is known to be associated with zoonotic potential (16). Not enough is currently known about the pathogenesis and zoonotic relevance of bovine or porcine chlamydioses, as there are virtually no reliable data on cases of zoonotic transmission or on possible modes of transmission to man. There have been reports of concentrations of Chlamydia infections in some dairy cattle units (17, e19, e20) and it has been postulated that persons in direct contact with infected animals might be at increased risk (e17).

Current studies on pathogenesis in man and animals allow the conclusion that chlamydial infections can induce acute clinical pictures, and the disease may even be fatal. However, it may be assumed that, in most cases, the disease is chronic and persistent, due to recurrent infections or the reactivation of infection already present (18, 19). It is not yet possible to evaluate the consequences for the patient and for the economic burden on the health care system (costs of treatment of infected patients) or on agriculture (loss of production, infected stocks). In vitro data allow the conclusion that both the phenomenon of chlamydial persistence and the extraordinary ability of the pathogen to manipulate the host cells appear to play essential roles in the pathogenesis of chlamydioses (20, 21). This hypothesis must be further verified in vivo; animal models are essential.

Status and perspectives of diagnostic testing

Laboratory diagnostic testing of chlamydial zoonosis pathogens is unsatisfactory—both in human and in veterinary medicine—as there is no commercially available test procedure which is both sensitive and species-specific.

Species-specific pathogen detection is still largely restricted to specialized laboratories. Cell culture of Chlamydiae is extremely difficult for some species. It is only feasible as a method of diagnostic testing in specialized laboratories with extensive practical experience.

Antigen ELISA tests for *Cp. psittaci* or *Cp. abortus* in human medicine are currently not commercially available. If antigen ELISAs are used for screening for the whole family of the Chlamydiaceae, it must generally be borne in mind that these tests are inferior to both detection in culture and the PCR with respect to sensitivity and specificity (22) and that this can often lead to misinterpretation of the findings.

The results of serological methods are of limited reliability, unless paired serum samples of patients are examined with species-specific procedures. These procedures are mostly for the detection of antibodies to *C. trachomatis*. Methodological problems remain to be solved in the detection of antibodies to *Cp. pneumoniae* or *Cp. psittaci*. No methods are available for the detection of antibodies to other Chlamydiae.

Rapid direct detection from clinical samples has only been possible since the introduction of DNA-based detection methods in the 1990s. Established and validated tests have now been developed on the basis of conventional (23) or real time PCR (16). These tests are mostly more sensitive than detection in culture; the specificity is about the same. PCR kits for zoonotic Chlamydiae are not yet commercially available.

A further development in Chlamydia testing has been the use of the DNA microarray test (AT) (24), which can be used for the detection and differentiation of 9 Chlamydia species. This technique permits the identification of the exact nucleotide sequence of a genomic target region and thus the recognition and differentiation of closely related species. In a recent validation study, a total of 339 samples, including 293 clinical samples from veterinary and human medicine, were tested for Chlamydiae in the AT test (e21). The performance parameters for the microarray test were good in comparison with a real time PCR protocol, conventional PCR tests, immunohistochemistry, and other detection methods for Chlamydiae. Moreover, this test is capable of detecting mixed infections with two or more different Chlamydia species. The sensitivity was similar to that of the real time PCR and better

than that of conventional 16S PCR and other methods compared. The study results indicate that the AT should be suitable for routine diagnostic testing.

It has been found to be very useful in practice to contact reference or expert laboratories about cases which are difficult to diagnose.

Therapeutic problems

Acute Chlamydia infections are normally treated with macrolides, tetracyclines, or quinolones. However, as mentioned above, Chlamydiae can develop persistent forms (so-called atypical reticular bodies). These are unaffected by normal standard therapies, even though there is no genetically determined resistance. This is referred to as phenotypic resistance (25). These persistent infections can lead to chronic clinical courses, as are observed in both respiratory and genital Chlamydia infections, coupled to major medical problems.

Research work

Morbidity and mortality from chlamydial diseases are of major global significance. Current knowledge on the pathogenesis and, in particular, the state of development of diagnostic testing and therapy, does not do justice to this state of affairs. The zoonotic potential of the pathogen is an important and current challenge for research and practice in human and veterinary medicine. In the view of the authors, the recently formed German national working group on the theme "Zoonotic Chlamydiae—models for chronic and persistent infections in man and animals" will address the currently most important and urgent issues. The main objectives are as follows:

- Performance of a clinical study to evaluate the possible link between exposure to zoonotic Chlamydiae and acute or chronic pulmonary inflammation;
- Development of an infection model in a large animal to clarify the pathways of zoonotic transmission, together with the molecular mechanisms of pathogenesis and the host immune response;
- Evaluation of new DNA microarray-based diagnostic procedures for the direct identification and genotyping of Chlamydiae in clinical samples;
- Development in the cell culture model of new approaches for the antibiotic therapy of persistent Chlamydia;
- Evaluation of the zoonotic potential of different Chlamydia species by compiling the results of all activities of the working group related to pathological mechanisms, epidemiology and new therapeutic strategies.

It is expected that the new German research association mentioned above will be the source of important new impulses towards improvements in the diagnostic testing and therapy of animal and human diseases from Chlamydiae.

Conflict of interest statement
The authors declare that there is no conflict of interest in the sense of the guidelines of the International Committee of Medical Journal Editors.

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